Distinct HLA-C/KIR Genotype Profile Associates with Guttate Psoriasis

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Psoriasis is a multifactorial disease with a strong genetic background. It associates strongly to HLA-Cw*0602. HLA-C interacts with killer immunoglobulin-like receptors (KIR) on natural killer (NK) and some natural killer-T (NKT) cells. KIR's function is triggered by specific binding to HLA ligands, which depends on the amino acid 80 of the MHC class I α-chain. This permits classifying all HLA-C alleles into two functional groups: asparagine (N80) or lysine (K80) carrying alleles. Psoriasis patients recruited at disease onset were categorized as guttate, vulgaris without arthropathy and vulgaris with arthropathy plus skin lesions. Patients and carefully matched controls were genotyped for position 80 of *HLA-C* and for KIR. Based on possible HLA/KIR combinations, individuals were classified according to expected NK/NKT cell responses: balanced (B), excess inhibition (EI), excess activation (EA), or undetermined (U). HLA-Cw6 and position 80 genotyping associated strongly to disease, whereas KIR2DS1 associated weakly. Individuals of the U and EI classes were more common among guttate psoriasis patients, which related to HLA-Cw*0602 status. These results suggest that different levels for NK/NKT cell activation thresholds, not only reduction, contribute to immune deregulation in psoriasis. In the guttate phenotype, balanced HLA-C/KIR interactions might be altered by the presence of concomitant streptococcal infections.

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Psoriasis is a multifactorial skin disease with autoimmune features that affects approximately 2% of the Caucasian population. Psoriatic skin lesions appear as red scaly plaques with an underlying inflammatory infiltrate involved in both lesion initiation and maintenance (Hellgren, 1967). Psoriasis vulgaris (PsV) is the most common clinical form of the disease. It manifests with plaques located most often on the scalp and extensor surfaces. Guttate psoriasis (PsG) presents with acute and small drop-like lesions scattered over the trunk, usually after an upper respiratory tract infection. A significant proportion of patients develop concurrent arthritis (PsA), which is potentially incapacitating (Christophers and Kiene, 1995; Asumalahti *et al*, 2002; Holm, 2005).

The cause of the disease and its subtypes is still unknown, but several studies point to a strong genetic predisposition interacting with triggering environmental factors. The search for genetic determinants of psoriasis has been intense, especially in the MHC region of chromosome 6p21.3 where the psoriasis susceptibility locus (PSORS)1 locus resides. This region is thought to harbor a major psoriasis gene(s) and has been thoroughly investigated (Balendran *et al*, 1999; Oka *et al*, 1999; Nair *et al*, 2000). Several PSORS1 candidate genes were proposed and tested in association studies (*STG, CDSN, PSORS1C1-3, HCR, TCF19, POU5F1, HLA-C*) (Jenisch *et al*, 1999; Enerback *et al*, 2000; Gonzalez *et al*, 2000; Teraoka *et al*, 2000; O'Brien *et al*, 2001; Asumalahti *et al*, 2002; Chang *et al*, 2003; Holm *et al*, 2003; Sanchez *et al*, 2004; Carlen *et al*, 2005). The earliest association reported in psoriasis for any of these candidate genes was to *HLA-C* (McMichael *et al*, 1978). Even though HLA-Cw*0602, an allele of the *HLA-C* gene, remains the factor with the strongest association to psoriasis, its role is disputed with arguments such as: not all HLA-Cw*0602-positive individuals develop psoriasis, more than 30% of psoriasis patients are HLA-Cw*0602 negative, haplotypes carrying HLA-Cw*0602 associate more strongly to psoriasis than the allele itself, and other candidate variants reach the same level of association as HLA-Cw*0602 (Trembath *et al*, 1997; Jenisch *et al*, 1999; Asumalahti *et al*, 2000; Nair *et al*, 2000; Allen *et al*, 2001; Chia *et al,* 2001; Asumalahti *et al,* 2002). These arguments are, however, based on methods that bear the expectation of simple Mendelian inheritance patterns, which in the case of the PSORS1 locus, are unlikely to accommodate for the likely effect of other candidate genes in the region and of genes in other genomic locations (PSORS2–PSORS9) (Wain *et al,* 2004). Psoriasis is a multifactorial disease and, as such, *HLA-C* cannot be disregarded simply because of non-Mendelian behavior. HLA-Cw*0602 is indeed present in about 60% of the psoriasis patients compared with 10% of the controls in the Swedish population (Enerback *et al*, 1997).

Abbreviations: B, balanced; CI, confidence interval; EA, excess activation; EI, excess inhibition; K, lysine; KIR, killer immunoglobulin-like receptor; MIC, MHC class I chain-related; N, asparagine; NK, natural killer; NKT, natural killer-T; PsA, psoriasis arthritis; PsG, guttate psoriasis; PsV, psoriasis vulgaris; OR, odds ratio; PSORS, psoriasis susceptibility locus; U, undetermined

Relative to other PSORS1 candidates, HLA-Cw*0602 consistently shows the strongest association to disease in various populations, (Economidou, 1985; Nakagawa *et al*, 1991; Enerback *et al*, 2000; O'Brien *et al*, 2001; Asumalahti *et al*, 2002; Kundakci, 2002; Tsai *et al*, 2002; Holm *et al*, 2003). Furthermore, homozygotes for this allele appear to have a higher risk of developing psoriasis than heterozygotes (Gudjonsson *et al*, 2003). In addition, altered immune responses seem to be central to psoriasis pathogenesis, which provides a ground for studying HLA-C's involvement in this phenotype. The contribution of HLA-C to psoriasis should be studied from the perspective that its effect is important but partial, it associates more with some clinical features (young age, PsG), and that it might interact with other candidate genes in this and/or other regions.

HLA-C is recognized by natural killer and natural killer-T (NK/NKT) cells through the killer immunoglobulin-like receptors (KIR) (Colonna *et al*, 1993; Mandelboim *et al*, 1996; Winter *et al*, 1998). These and other NK/NKT cell receptors enable the immune system to assess the type and level of HLA class I expression on target cells (Algarra *et al*, 2004). Cells with altered HLA class I expression induce NK/NKT cell activation (Bottino *et al*, 2004). Miscommunication between HLA-C and KIR is likely to initiate inappropriate immune responses. Interestingly, the number of circulating NK/NKT cells appears to be reduced in psoriasis, as in other autoimmune disorders, suggesting a pathogenic role for these cells (Cameron *et al*, 2002). In fact, NK/NKT cells, a subpopulation of T cells that also carry KIR, are found in chronic psoriatic plaques and are known to induce similar lesions in severe combined immunodeficient (SCID) mice (Nickoloff, 1999; Bonish *et al*, 2000; Gilhar *et al*, 2002). In addition, recent data show that activated T cells are required for development of psoriatic lesions in K5.Stat3C mice (Sano *et al*, 2005).

So far, the KIR locus on chromosome 19q13.4 is known to contain 16 KIR genes and pseudogenes (Hubbard *et al*, 2002). KIR are activating or inhibitory depending on their structure. The activating receptors carry a short (S) cytoplasmic tail and the inhibitory receptors carry a long (L) cytoplasmic tail. Upon engagement by MHC class I molecules, KIR(L) regulate NK/NKT cell cytotoxicity by antagonizing the activating signals from KIR(S)-activating receptors (Bottino *et al*, 2004). KIR are able to distinguish between different HLA-A, B, and C allotypes according to the allelic residue at position 80 of the MHC class I α1 helix. Group 2 HLA-C alleles carrying a lysine (K80) at this position (HLA-Cw2, 4, 5, 6, and 15) are recognized by KIR2DL1, and KIR2DS1. Group 1 alleles, carrying an asparagine (N80), are recognized by KIR2DL2 and KIR2DS2 (HLA-Cw1, 3, 7, and 8) (Mandelboim *et al*, 1996; Winter and Long 1997; Boyington *et al*, 2001). Since the number of genes at the KIR locus varies between individuals from 7 to 14 genes (Uhrberg *et al*, 1997), and since the MHC and KIR loci reside on different chromosomes, individuals can inherit both the ligand and the receptor, only the ligand, or only receptor. These HLA/KIR combinations could thereby lead to differences in NK/NKT cell activation thresholds. Recent studies show the association of KIR2DS1 to psoriasis vulgaris (Luszczek *et al*, 2004; Suzuki *et al*, 2004), and of KIR2DS1 and KIR2DS2 to psoriasis arthritis (Hewett *et al*, 2002; Nelson *et al*, 2004).

The frequency distributions for the various HLA/KIR combinations among patients with PsG, PsV, and PsA were compared with those found in the control group. All individuals were classified according to the expected net biological effect for such combinations. Significantly less PsG individuals were classified as having undetermined (U) HLA-C/KIR combinations. Also, among these patients there were more individuals classified as having an increased potential for NK/NKT cell inhibition. A trend for increased potential for activation was found among PsA patients.

Results

The distributions of genotypes for HLA-Cw*0602, KIR2DL1, KIR2DL2, KIR2DS1, KIR2DS2, and KIR2DS4 in 396 psoriatic patients and 372 matched controls were identified. Genotypes for the codons 73, 77, and 80 of the *HLA-C* gene, and combinations of codon 80 variants with their functionally related KIR genes were also studied.

All patients and controls were recruited from the Stockholm area, and had similar gender and age distributions. But age differences were detected between phenotypes. PsG patients were significantly younger (mean = 32.8 y) than vulgaris (mean = 42.3 y) and psoriasis arthritis patients (mean = 45.8 y) ($p = 10^{-6}$). Proportions of males and females were comparable across phenotypes and among controls, although a trend for more women in the arthritis group was observed.

HLA-C typing

*HLA-Cw*0602 association to psoriasis varied depending on phenotype* The background level of HLA-Cw*0602 positivity in our sample was 10.5%. Patients carried this allele in a significantly higher proportion (38%, odds ratio (OR) = 5.23, confidence interval (CI) [3.54–7.73], $p = 4.07 \times 10^{-19}$) but the differences were more pronounced for patients classified within certain phenotypes (Table I). Remarkably, HLA-Cw*0602 was found in 73% of guttate patients (OR = 22.70, CI [12.44–41.42], $p = 2.69 \times 10^{-28}$).

Genotypes at codon positions 73, 77, and 80, as determined by pyrosequencing, were found to be at expected frequencies in the control population. Positions 77 and 80 were in linkage disequilibrium ($D' = 0.95$, CI [0.91–0, 97], $r^2 = 0.69$), whereas position 73 appeared to segregate independently. Position 73 was associated with PsV diagnoses, whereas it showed an inversed pattern of association to PsA (Table II). Genotypes at position 77 and position 80 were significantly associated with disease status. There was similar risk for position 77 heterozygote and TT homozygote PsG individuals (OR = 12.6 *vs* OR = 11.1), whereas association to either genotype was much lower for PsA and PsV (Table II). Position 80 TT individuals were significantly more common among PsG individuals but the level of association to psoriasis was about three times lower than that for the heterozygote genotype (OR = 3.9 *vs* OR = 13.1).

Estimated haplotypes between positions 77 and 80 occurred at the following frequencies: 7.3% for 77C–80T, 39.5% for 77T–80T, and 52.5% for 77C–80G. Haplotypes 77C-80T and 77T-80T were significantly associated with

Table I. HLA-Cw*0602 association to psoriasis according to phenotype

Category	Frequency	OR (CI)	p-value[a]
Guttate	72.73% (56/77)	22.70 (12.44–41.42)	2.69×10^{-28}
PsA	24.62% (16/65)	2.78 (1.44–5.35)	0.0037
Vulgaris	30.54% (73/239)	3.74 (2.43–5.76)	4.58×10^{-7}
Patients total	38.06% (145/381)	5.23 (3.54–7.73)	4.07×10^{-19}
Controls	10.51% (39/371)	—	—

[a]Determined by Fisher's exact test.
OR, odds ratio; CI, confidence interval; PsA, psoriasis arthritis.

psoriasis ($\chi^2 = 15.86$ $p = 6.82 \times 10^{-5}$, and $\chi^2 = 7.67$ $p = 0.0056$, respectively).

KIR2DS1 associates to PsV The proportion of individuals with activating KIR2DS1 receptors was significantly higher among patients than controls (OR = 1.47, CI [1.10–1.96], p = 0.010), particularly in PsV patients (OR = 1.48, CI [1.06–2.05], p = 0.023). There was also a trend towards a higher KIR2DS1 frequency among PsA patients (Table III). The other KIR receptors considered here did not associate to disease.

The most common KIR combinations found in this material are presented in Table IV. None of them associated with disease status.

PsG patients differed from other phenotypes when classified according to functional expectations for HLA-C position 80/KIR genotype combinations The proportions of individuals in the balanced (B)-, excess inhibition (EI)- or excess activation (EA)-predicted "functional" classes were similar between controls and patients as a whole. Individuals within the EA class were under-represented among patients and controls in general and across phenotypes, although there was an increased proportion of EA individuals among PsA patients (4% *vs* 1.2%, $\chi^2 = 1.67$, DF = 1, P = NS) (Fig 1).

PsG patients showed a distinctive profile compared with the other phenotype groups ($\chi^2 = 22.14$, DF = 9, p = 0.01). They had similar proportions of B and EA individuals as controls, more EI individuals (47.44% *vs* 31.93%, $\chi^2 = 6.4$, DF = 1, $p_{corrected} = 0.048$), and significantly less individuals of the U class (3.85%, *vs* 21.01% $\chi^2 = 12.81$, DF = 1, $p_{corrected} = 0.0004$). The proportion of individuals classified as having potentially balanced NK/NKT cell responses was similar between all groups, but about one-third of the guttate patients with this profile had a concomitant streptococcal throat infection at psoriasis onset (data not shown).

Individuals carrying HLA-Cw*0602 were more likely to be classified as EI, whereas most U individuals lacked this allele (Fig 2).

When stratifying patients according to age of disease onset into type I or type II, no differences in HLA-C/KIR distributions were observed (data not shown).

Discussion

In this study, the relationship of *HLA-C* with psoriasis was explored from the perspective of gene–gene interaction. Accordingly, the KIR locus was considered the primary interacting partner to *HLA-C*. The basis for this was the emerging concept that HLA-C/KIR molecular interactions depend on a limited number of conditions: the amino acid

Table II. Odds ratios (OR), confidence intervals (CI), and p-values for the frequencies of variants at codon position 73, 77, and 80 of HLA-C in different psoriasis phenotypes

	Guttate		PsA		PsV		All phenotypes	
Position	OR (CI)	p_c-value[a]	OR (CI)	p_c-value[a]	OR (CI)	p_c-value[a]	OR (CI)	p_c-value[a]
73								
CC	1.29 (0.49–3.44)	NS	0.55 (0.27–1.10)	NS	2.23 (1.35–3.85)	0.009	1.43 (0.92–2.23)	NS
CT	2.52 (1.08–6.07)	NS	0.44 (0.23–0.85)	0.042	1.21 (0.70–2.07)	NS	1.08 (0.70–1.65)	NS
77								
CT	12.2 (3.57–50.14)	6×10^{-8}	0.69 (0.36–1.32)	NS	1.29 (0.86–1.93)	NS	1.50 (1.05–2.14)	NS
TT	12.3 (4.46–39.18)	6.7×10^{-6}	1.91 (0.97–3.76)	NS	1.94 (0.87–2.40)	NS	1.94 (1.25–3.00)	0.01
80								
GT	14.2 (5.37–41.34)	0.002	1.21 (0.69–2.14)	NS	1.94 (1.31–2.89)	0.003	2.31 (1.65–3.25)	1.2×10^{-5}
TT	11.7 (2.65–14.12)	0.039	0.73 (0.30–1.71)	NS	1.85 (1.12–3.06)	0.045	1.7 (1.10–2.68)	0.031

[a]Determined by Fisher's exact test.
p_c, corrected p value; PsV, psoriasis vulgaris; PsA, psoriasis arthritis; NS, not significant.

Table III. Genotype frequencies of KIR genes in patients and controls

KIR gene	Controls	Guttate	OR (CI)	p-value[a]	PsA	OR (CI)	p-value[a]	Vulgaris	OR (CI)	p-value[a]	Patients total	OR (CI)	p-value[a]
2DL1	97.6% (362/372)	98.8% (79/80)	1.96 (0.25–15.73)	NS	90.7% (68/75)	0.24 (0.09–0.67)	NS	95.4% (230/240)	0.57 (0.23–1.43)	NS	95.2% (377/396)	0.52 (0.23–1.17)	NS
2DL2	49.3% (182/372)	58.2% (46/79)	1.43 (0.88–2.34)	NS	53.3% (40/75)	1.17 (0.71–1.93)	NS	50.4% (117/232)	1.05 (0.75–1.45)	NS	52.6% (203/386)	1.14 (0.86–1.52)	NS
2DS1	42.2% (152/372)	48.8% (39/80)	1.30 (0.80–2.12)	NS	54.7% (41/75)	1.65 (1.00–2.72)	0.0555	51.9% (123/237)	1.48 (1.06–2.05)	0.0234	51.8% (203/392)	1.47 (1.10–1.96)	0.0105
2DS2	46.8% (173/372)	53.8% (43/80)	1.32 (0.82–2.15)	NS	54.7% (41/75)	1.37 (0.83–2.26)	NS	45.0% (108/240)	0.93 (0.67–1.29)	NS	48.6% (192/395)	1.08 (0.81–1.43)	NS

[a]Determined by Fisher's exact test.
KIR, killer immunoglobulin-like receptor; OR, odds ratio; CI, confidence interval; NS, not significant.

present at position 80 of HLA-C (which defines the subset of KIR that can bind to this protein), the expression of specific KIR genes, the number of HLA class I molecules expressed on target cells, and the expression of other NK/NKT cell receptors able to sense HLA levels (Mandelboim *et al*, 1996; Uhrberg *et al*, 1997; Winter *et al*, 1998; Boyington and Sun, 2002; Bottino *et al*, 2004). An arbitrary classification of study individuals into potential "functional" classes was established. Four classes were defined as representing the likely functional outcome of HLA-C/KIR interactions, assuming expression of all alleles involved and no interference from other gene products (Table V). These classes were compared between patients and controls under the hypothesis that, if interactions between these two molecules were relevant to psoriasis, a non-random distribution of response profiles would be found.

The main effects of HLA-Cw*0602 and of the KIR genes considered in this study agreed with association measurements reported previously in different studies (Enerback *et al*, 2000; O'Brien *et al*, 2001; Asumalahti *et al*, 2002; Holm *et al*, 2003). HLA-Cw*0602 strongly associated to psoriasis, especially to PsG. Significant association was also observed for early-onset psoriasis (<25 y of age), which correlated with a large proportion of PsG individuals of young age at disease onset (data not shown) (Enerback *et al*, 1997; Mallon, 2000; Gudjonsson *et al*, 2002). Genotypes at positions 77 and 80 also associated strongly to psoriasis, following the same pattern as HLA-Cw*0602 in relation to phenotype and age of onset. In contrast, position 73 was only slightly associated with PsG and PsA diagnoses, whereas it showed an inverse pattern of association to PsV.

In this study, KIR2DS1 associated significantly to psoriasis, particularly to PsV, and showed a trend in PsA. Previous studies report excess of individuals with activating receptors that lack a ligand for the corresponding inhibitory receptor among PsV patients (Luszczek *et al*, 2004; Suzuki *et al*, 2004) and among PsA patients (Hewett *et al*, 2002). These findings support the concept that inadequate control of NK/NKT cell activation contributes to psoriasis pathogenesis.

A number of studies report that particular HLA Class I/KIR combinations contribute to disease. For example, HIV infection in people carrying HLA-Bw4-80Ile and KIR3DS1 progresses slower to AIDS; type I diabetes patients more often carry KIR2DS2 and its ligand HLA-C-Asn80, which RA patients with vasculitis also carry, in the absence of the corresponding inhibitory receptor (Venter *et al*, 2001; Hewett *et al*, 2002; van der Slik *et al*, 2003). These studies focused on determining the proportions of individuals carrying certain KIR in the presence or absence of particular ligand HLA class I molecules. Using a more robust approach, this study predicted the likely biological effects of HLA/KIR combinations based on limited assumptions, and classified study individuals accordingly. This method revealed that individuals within the EA class, i.e., individuals with HLA-C/KIR combinations favoring NK/NKT cell activation, were a minority among patients as well as controls. A trend for higher proportions among PsA individuals, however, was also found. This result along with previous indications that inhibitory receptors have higher affinity for their ligand than activating receptors (Winter *et al*, 1998), and that placental

Table IV. Distribution of KIR combinations in patients and controls (n)

KIR[a]	Control	Guttate	PsA	Vulgaris	Patients total
PNNN	112	16	16	64	96
PNPN	68	15	18	49	82
PPNP	82	21	15	41	77
PPPP	72	19	18	57	94

[a]The KIR genes are presented in following order: 2DL1, 2DL2, 2DS1, and 2DS2.
KIR, killer immunoglobulin-like receptor; P, present; N, absent; PsA, psoriasis arthritis.

inhibition of NK/NKT cell activation is crucial during pregnancy (Hiby *et al*, 2004) suggest that profiles favoring lower NK/NKT cell activation thresholds would be maintained at low frequencies in populations. Activation in this system occurs naturally when HLA-C expression is reduced, which is a common feature in viral infections and cancer. But, in theory, direct engagement of activating KIR or manipulation of their signalling pathways by infectious agents could lead to disease. This study also found that PsG had a singular predicted responsiveness profile. Among these patients, there was a higher proportion of EI, and a reduced proportion of U profiles. Individuals of the EA class were more common among PsA patients, which is in agreement with the findings reported above in relation to KIR2DS1. A large proportion of HLA-Cw*0602-positive EI class PsG individuals was found, whereas this allele was significantly uncommon among U class individuals. Additionally, PsG patients belonging to class B more often reported a concomitant throat infection at disease onset, confirmed as streptococcal by swab tests.

These findings suggest that inadequate communication between HLA-C and KIR contributes to psoriasis pathogenesis and that HLA-Cw*0602 is an important player, especially in PsG and likely in relation to streptococcal infection in young individuals (Enerback *et al*, 1997; Allen *et al,* 2005). Since the NK/NKT cell response profiles found in this population were robustly defined by zygosity at position 80 regardless of the HLA-C alleles present, contribution by alleles other than HLA-Cw*0602 is expected. Studies that report weak association to HLA-Cw6 might benefit from incorporating KIR into their analyses, thus accounting for the effect of other HLA-C alleles. It seems intuitive that lower NK/NKT cell activation thresholds contribute to pathogenesis (Hewett *et al*, 2002); in contrast, our finding that more PsG patients fell into the EI class is somewhat intriguing in relation to psoriasis pathogenesis.

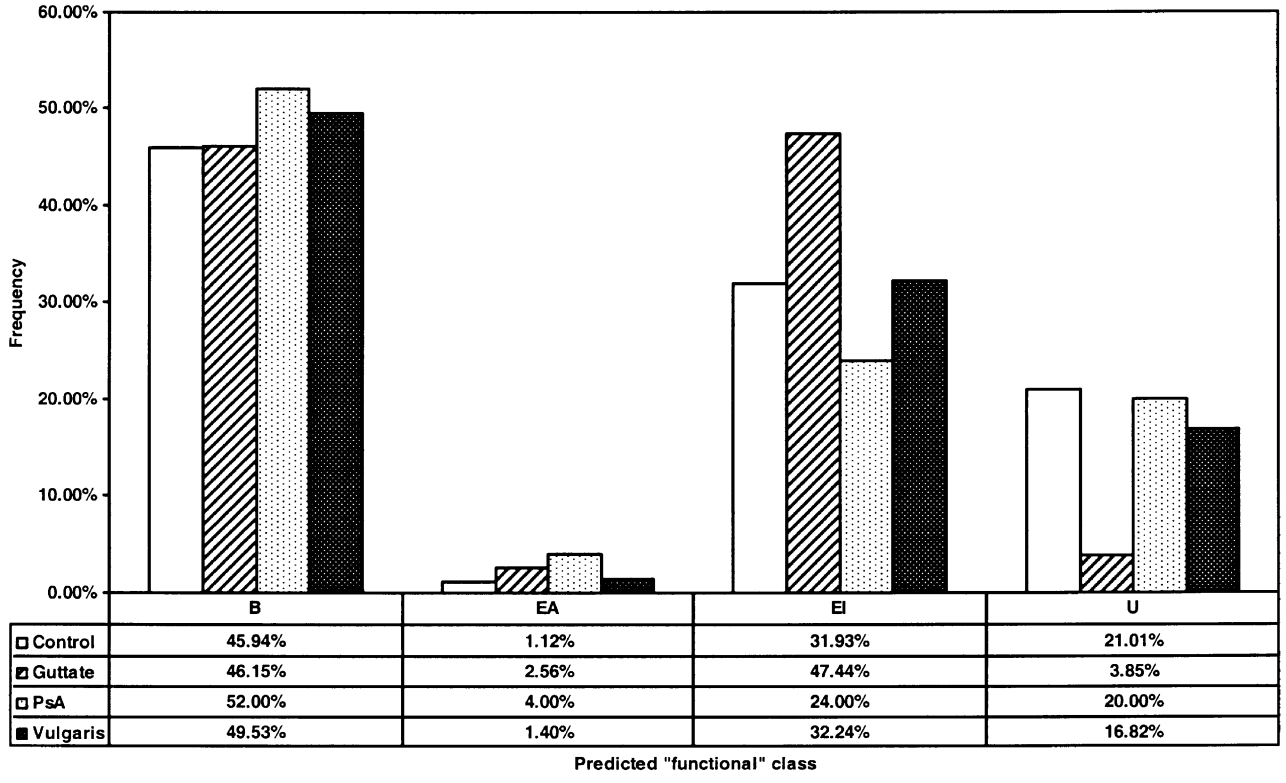
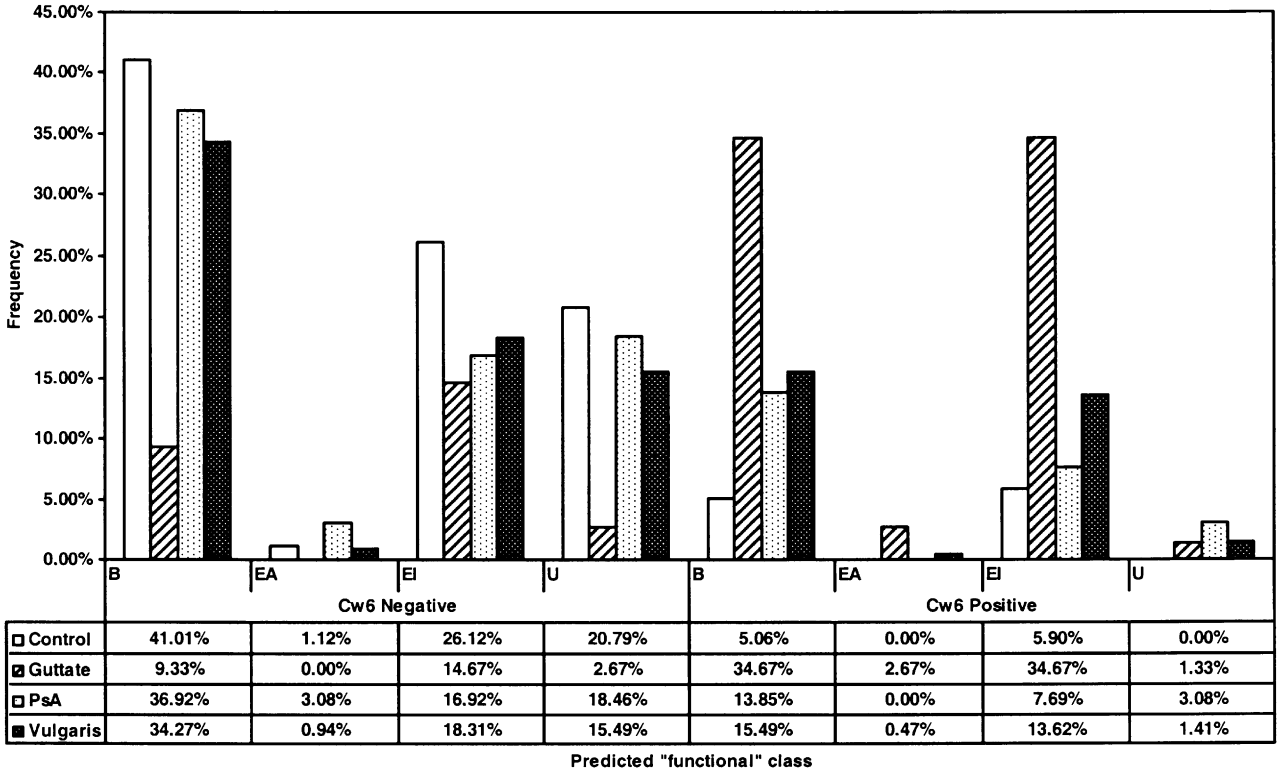


Figure 1
Distribution of predicted functional classes among guttate psoriasis (PsG), psoriasis arthritis (PsA), and psoriasis vulgaris (PsV) patients and among controls. HLA-C/KIR genotype combinations according to phenotype.



	Cw6 Negative				Cw6 Positive			
	B	EA	EI	U	B	EA	EI	U
Control	41.01%	1.12%	26.12%	20.79%	5.06%	0.00%	5.90%	0.00%
Guttate	9.33%	0.00%	14.67%	2.67%	34.67%	2.67%	34.67%	1.33%
PsA	36.92%	3.08%	16.92%	18.46%	13.85%	0.00%	7.69%	3.08%
Vulgaris	34.27%	0.94%	18.31%	15.49%	15.49%	0.47%	13.62%	1.41%

Figure 2
Distribution of proportions of HLA/KIR (killer immunoglobulin-like receptor) combinations in patients with guttate, vulgaris, and arthritis psoriasis and controls divided by HLA-Cw*0602 status (N, negative, P, positive). HLA-C/KIR genotype combinations according to phenotype and HLA-Cw6 status.

Does the contribution of this profile to psoriasis result from a reduced ability to overcome inhibition when activation is required? Does it result from a direct pathogenic effect of the inhibitory process? Also intriguing was the presence of similar proportions of B class individuals across phenotypes and in controls. There might be different ways to produce imbalance in the NK/NKT cell activation process. It is possible that this system is a target for different triggering factors, such as infection (streptococcus, staphylococcus, other), pharmaceuticals, immunomodulatory molecules, etc. During infection for example, the lack of NK/NKT cell responsiveness could be advantageous to the agent in ways that favor psoriasis development as a by-product of immune deregulation. In this regard, it is important to mention that bias towards T_{H1} responsiveness is a hallmark of psoriasis, and that NK/NKT cells observed in this disease (Nickoloff, 1999; Bonish *et al*, 2000; Cameron *et al*, 2002) could be a source of T_{H1}cytokines. A T_{H1}-like cytokine secretion pattern (IFN-γ, TNF-α, and IL-6) was reported for NK/NKT cells upon engagement of activating receptors by HLA-C (Barakonyi *et al*, 2004). The fact that infections might trigger disease development does not imply that psoriasis patients are more susceptible to infection. It may suggest that individuals with inherently frail NK/NKT cell regulation are more prone to psoriasis. Remarkably, a third of the guttate patients with predicted balanced responses in this study had a concomitant streptococcal throat infection, suggesting that this factor might take them off balance, whereas it might further contribute to already imbalanced HLA-C/KIR interactions in other patients. Since our patients and controls are being prospectively followed, it is of interest to determine whether worsening of symptoms in patients or occurrence of psoriasis among controls correlates with the external triggers that are being considered in this cohort.

It is not clear as to what is responsible for the NK/NKT cell imbalance in U class individuals; however, cross-reactivity between KIR and HLA-C groups has been described. The model proposed here is assumed to be incomplete since it does not consider other known interacting partners. It is likely that other receptors and their ligands need to be included into the analysis before a more complete picture of the decision-making process leading to NK/NKT cell activation in psoriasis is developed. Since the HLA/NK cell receptor system senses quantitative differences in HLA class I expression, it is crucial to determine whether expression of both receptors and ligands (e.g., HLA-E) occur at proportions corresponding to the ones expected from the genotypic patterns. In other words, to study whether the potential NK/NKT cell responses in a given individual depend on an expression threshold. It is possible that differential allelic expression contributes to fine tuning the events leading to NK/NKT cell activation. Therefore, determining allelic expression at the RNA as well as the protein levels is essential in understanding the events involved in this interaction. Also environmental triggers that might bypass or modify HLA/KIR signalling, in favor of activation or inhibition under different pathological contexts, need to be determined.

The KIR locus was investigated in this study because these receptors are known functional interacting partners of HLA-C molecules. Indeed, an association between HLA-C/

Table V. Primers used for the amplification of HLA and KIR, expected amplicon size and annealing temperature

Primer	Sequence	Size (bp)	Temp (°C)
Phototyping			
HLA-Cw6 (127)	5′-GGT CGC AGC CAT ACA TCC A-3′	297	65
HLA-Cw6 (367)	5′-TAC TAC AAC CAG AGC GAG GA-3′		
DRB1 F[a]	5′-TGC CAA GTG GAG CAC CCA A-3′	796	—
DRB1 R[a]	5′-GCA TCT TGC TCT GTG CAG AT-3′		
KIR2DL1F	5′-TGG ACC AAG AGT CTG CAG GA-3′	330	63
KIR2DL1R	5′-TGT TGT CTC CCT AGA AGA CG-3′		
KIR2DL2F	5′-CTG GCC CAC CCA GGT CG-3′	173	62
KIR2DL2R	5′-GGA CCG ATG GAG AAG TTG GCT-3′		
KIR2DS1F1	5′-CTT CTC CAT CAG TCG CAT GAA-3′	102	64
KIR2DS1F2	5′-CTT CTC CAT CAG TCG CAT GAG-3′		
KIR2DS1R	5′-AGA GGG TCA CTG GGA GCT GAC-3′		
KIR2DS2F	5′-TTC TGC ACA GAG AGG GGA AGT A-3′	173	63
KIR2DS2R	5′-AGG TCA CTG GGA GCT GAC AA-3′		
Pyrosequencing			
HLA-C.F	5′-AGCGAGGKGCCCGCCCGGCGA-3′	918	67
HLA-C.R	5′-GGAGATGGGGAAGGCTCCCCACT-3′		
HLA-C.FN	5′-NNNNNNNNNCCCGCCCGGCddG-3′	919	67
HLA-C.RN	5′-NNNNNNNNNNAAGGCTCCCCddA-3′		
Pos80.F	5′-(Biotin)TCGACAGCGACGCCGCGAGTCC-3′	162	65
Pos80.R	5′-CCTCCGCTCTGGTTGTAGTAGCCGCG-3′		

[a]Internal control included in all reactions but for pyrosequencing. Primers are specific for a 796 bp amplicon from the third exon of DRB1.
KIR, killer immunoglobulin-like receptor; N, asparigine.

KIR combinations and psoriasis was found, supporting an immunity-based theory of psoriasis. This provides the possibility that other proteins known to be active in the immune system can be included in the model. An important assumption of such a broad model is that molecular interactions occurring in multiple ways may lead to disease outcome. This open model, more akin to observed biological networks, allows for the inclusion of other members of the PSORS1 region. For example, MIC molecules have previously been shown to associate with psoriasis. These molecules are in fact recognized by NK/NKT cells as their expression seems to be triggered by low levels of HLA class I expression (Cheng *et al*, 2000; Dunn *et al*, 2000). It will therefore be important to determine whether HLA-C and MIC, and perhaps other immune molecules interact in psoriasis pathogenesis.

Materials and Methods

Study population A total of 396 psoriasis patients and 372 controls were investigated in this study. The patients were recruited within 1 y of disease onset. Each patient was thoroughly examined by a dermatologist. Whenever psoriasis arthropathy was suspected, a rheumatologist assessed clinical features to ensure diagnosis. PsV without joint symptoms was diagnosed in 241 patients, PsG for 80 patients, and PsV with concomitant arthropathy in 75. A few patients manifested mild joint symptoms but a clear acute onset of guttate lesions. They were classified within the guttate category for the present analysis purposes. The characteristics of the majority of patients are described in detail elsewhere (Mallbris, 2005). The mean age of onset was 41 y (females 41.85, males 39.78), with a range between 12 and 84 (females 12–84, men 13–80). The controls consisted of 214 females and 158 males and were selected from the Stockholm County as random samples by means of the population register, taking age, sex, and living district into consideration. Informed consent was given by all study participants. This study fulfilled the requirements of the Regional ethics Committee of Stockholm, Sweden, and was performed according to the Declaration of Helsinki.

Bioinformatics To ensure proper positioning of the primers used in this study, all known alleles of HLA-C and KIR were considered for sequence analysis (Bunce *et al*, 1995; Hewett *et al*, 2002; etokebe *et al*, 2003). Publicly available sequences for HLA-C and KIR (Robinson *et al*, 2005) were imported and assembled using the Pregap4 and Gap4 programs of the Staden package (Bonfield *et al*, 1998) and compared with each other. All primer sequences designed were compared with the human genome using BLAST. In addition, primers designed for HLA-C-related amplifications and sequencing were compared with SNP and STS databases to search for unknown SNP that could affect primer binding and amplification in this very polymorphic region.

HLA-Cw*0602 and KIR genotyping Genomic DNA was extracted from all individuals using standard conditions (Sambrook *et al*, 1989). For the detection of products from the KIR2DL1, KIR2DS1,

Table VI. Classification scheme of HLA-C/KIR genotype combinations and their potential NK cell response

		HLA-C position 80 amino acid							
		K (lysine)				N (asparagine)			
HLA-C position 80 amino acid	Corresponding KIR	L1/S1	L1/00	00/S1	00/00	L2/S2	L2/00	00/S2	00/00
K (lysine)	L1/S1	B							
	L1/00	B	EI						
	00/S1	B	B	EA					
	00/00	B	EI	EA	U				
N (asparagine)	L2/S2	B	EI	EA	B	B			
	L2/00	EI	EI	B	EI	B	EI		
	00/S2	EA	B	EA	EA	B	B	EA	
	00/00	B	EI	EA	U	B	EI	EA	U

The genotyping corresponding to the amino acid present at position 80 of HLA-C is identified as N or K. KIR receptor genotypes are identified as L1 (KIR2DL1), S1 (KIR2DS1, L2 (KIR2DL2) and S2 (KIR2DS2). Absent genotypes for KIR receptors are identified by 00. Columns represent the possible genotypic combinations for one copy of HLA-C, and the rows indicate the combinations for the second copy. Each cell identifies the theoretical NK cell responses expected for specific position 80 and KIR genotype combinations found at both HLA-C copies.
KIR, killer immunoglobulin-like receptor; B, balanced; EI, excess inhibition; EA, excess activation; U, undetermined.

KIR2DL2, and KIR2DS2 genes, previously published primer sequences were used (Hewett *et al*, 2002) (Table VI). Amplification was performed under standard conditions using Taq polymerase (Promega, Madison, Wisconsin) in a PTC-225 DNA Engine Tetrad (MJ Research, Waltham, Massachusetts). Briefly, 50–100 ng genomic DNA was used as a template in 10 μL PCR mixtures containing 100 nM of each specific primer, 50 mM of each internal control primer, 2 mM dNTP, 0.95% DMSO, 0.5 U of Taq polymerase, 67 mM Tris-HCl pH 8.8, 16 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 0.01% Tween-20, 0.1 mg per mL BSA. Temperature cycling consisted of the following: denaturation at 95°C for 2 min, five cycles of 20 s at 94°C, 30 s at 68°C, and 80 s at 72°C, followed by 30 cycles of 20 s at 94°C, 30 s at 62°C–65°C (Table VI), and 80 s at 72°C and a final elongation step at 72°C for 5 min. HLA-Cw*0602 status was determined as described previously (Bunce *et al*, 1995). Resulting products of predicted size were visualized under ultraviolet light after electrophoresis in 1.5% agarose gels containing ethidium bromide.

Pyrosequencing for *HLA-C* variants Genotyping of identified SNP specific for the three codons determining amino acids 73, 77, and 80 of HLA-C was carried out through Pyrosequencing in a 96-well format, according to the supplier's recommendations (Pyrosequencing, Uppsala, Sweden). HLA-C-specific amplicons were produced by a nested amplification protocol. First, an outer PCR using HLA-C-specific primers (HLA-CF, HLA-CR) was performed. To block amplification from closely related sequences this reaction was carried out in the presence of competitor primers (degenerated and dideoxy capped oligos, HLA-CFN, and HLA-CFR) (Etokebe *et al*, 2003) (Table VI). Temperature cycling conditions consisted of: 35 cycles of 20 s at 95°C, 60 s at 67°C, and 120 s at 72°C, followed by a final elongation step of 8 min at 72°C. These products were used as template for a nested PCR using primers pos80.F and pos80.R (Table VI). These primers were designed to recognize homologous sequences on most alleles. Care was taken to avoid polymorphic places that could bias amplification reactions. Temperature cycling conditions for the nested PCR were as follows: denaturation at 96°C for 1 min, five cycles of 25 s at 96°C, 45 s at 70°C, and 45 s at 72°C, followed by 21 cycles of 25 s at 96°C, 50 s at 65°C, and 45 s at 72°C, and an additional 11 cycles of 25 s at 96°C, 60 s at 55°C, and 120 s at 72°C. The forward primer was biotinylated at the 5′ end to allow capture using streptavidin-coated magnetic beads (Dynal Biotech, Oslo, Norway). Captured products were denatured, and the remaining single strands were annealed to the sequencing primer (pos80.R) (Table VI). Sequencing was performed on a PSQ96 Pyrosequencer using the reaction mixtures provided by the supplier (Pyrosequencing, Uppsala, Sweden). Reactions were followed in real time as luminous signals generated during nucleotide polymerization. These signals were automatically converted to genotypes by the PSQ HS96A 1.2 software. Data quality control was performed by direct inspection against all possible patterns expected for the three contiguous variations targeted in this study.

Classification of HLA-C/KIR combinations according to expected effects Activating and inhibitory KIR initiate their corresponding signalling cascades after binding to their specific ligands. For each ligand group, there is a corresponding set of one activating and one inhibitory receptor; group 2 (K80) alleles interact with KIR2DL1 and KIR2DS1, and group 1 (N80) with KIR2DL2 and KIR2DS2. In human populations, however, there are different proportions of individuals who carry ligand and receptor simultaneously, some ligand only and some receptor only. The predicted biological outcomes from these situations were defined in this paper as follows: B, if both inhibitory and activating HLA/KIR combinations for either or both ligand group(s) were present simultaneously; EI, if one or two inhibitory HLA/KIR combinations were present but activating HLA/KIR combinations were in minority or missing; EA, if one or two activating HLA/KIR combinations were present but inhibitory HLA/KIR combinations were in minority or missing; and undetermined (U) when no matching HLA/KIR combinations were detected (Table II). All combinations were based on zygosity information from position 80.

Statistics Hardy–Weinberg (HW) equilibrium and linkage disequilibrium (LD) tests were performed using the R language and program for statistical computing (Ihaka and Gentleman, 1996). Comparisons of proportions are presented with their corresponding OR and 95% CI. Their significance values were determined by Fisher's exact test. A significance level $\alpha = 0.05$ was the reference for most comparisons. Bonferroni correction was used to define significance levels when indicated. Haplotype estimation was performed using the partition/ligation method implemented in Haploview (Barrett *et al*, 2005).

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